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In Silico Docking Analysis of Phytochemicals from Orange Pith (Citrus Sinensis Albedo): Evaluating Binding Affinities to Human Salivary Amylase (PDB ID: 1SMD) for Diabetes Management

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ABSTRACT:

Around the world, diabetes mellitus is a major health issue associated with decreased quality of life, increased morbidity and mortality and increased burden of health care costs. Current diabetic therapies aimed at lowering blood sugar levels but did not address the root cause of diabetes mellitus as progression of insulin deficiency was not improved. Oral drugs for diabetes mellitus have many problems associated with treatment including loss of beta cell function and cause increase in impaired insulin secretion. This study focuses on exploring the efficacy of docking Orange Pith (Citrus Sinensis Albedo) to help lower the blood sugar of Diabetes Salivary. Using molecular docking, a total of 16 compounds from Orange Pith were subjected to in-silico docking analysis. The study reveals compelling results, with the top-ranking compound, Naringenin, exhibiting a strong binding affinity of -9.8 kcal/mol, followed by Luteolin -9.0 kcal/mol. The binding affinities of the remaining ligands, including Apigeninl and Epicatechin, varied between -8.8 and -8.7 kcal/mol. These findings shed light on the promising role of Orange Pith phytochemicals in hindering Salivary Alpha-Amylase (PDB ID: 1SMD), a key protein responsible for host entry. The study underscores the importance of further research and validation to develop effective therapeutic strategies against diabetes, emphasizing the potential of natural compounds as a resource for antiviral drug discovery. Moreover, molecular docking provides valuable insights into protein-ligand interactions, aiding in identifying potential candidates for experimental validation and drug development. Strict biosafety measures are imperative in handling this high-risk virus, and in silico approaches offer a safe and efficient means to explore potential inhibitors for the Diabetes.

KEYWORDS: *Diabetes, Orange Pith (Citrus Sinensis Albedo), Molecular Docking, Salivary Alpha-Amylase (PDB ID: 1SMD)*

1. INTRODUCTION

Diabetes is a growing global health crisis, affecting millions worldwide and significantly impacting public health, particularly in the Philippines and the United States. The disease, characterized by elevated blood glucose levels due to insulin resistance or deficiency, leads to severe complications such as cardiovascular diseases, kidney failure, and nerve damage. In the Philippines, diabetes prevalence is high, especially among older individuals, with contributing factors such as obesity and excessive consumption of refined carbohydrates. The COVID-19 pandemic further exacerbated the challenges faced by diabetic individuals, making them more vulnerable due to weakened immune systems. Current treatments primarily focus on lowering blood sugar levels but fail to address the underlying progression of insulin deficiency. Despite advancements in research, available anti-diabetic drugs still pose risks such as impaired insulin secretion, cardiovascular complications, and loss of beta-cell function. According to the World Health Organization, over 422 million people globally have diabetes, with an increasing trend over the past decades. The need for better management strategies and alternative therapeutic approaches is urgent.

Oranges, particularly their pith, offer promising potential in diabetes management due to their rich content of bioactive compounds. The pith contains pectin, a type of fiber that slows digestion and moderates blood sugar absorption, preventing sudden glucose spikes. Additionally, flavonoids and naringenin found in oranges enhance insulin sensitivity and reduce inflammation, supporting metabolic health. Oranges are also abundant in vitamin C, potassium, and folate, which contribute to improved blood sugar regulation, cholesterol reduction, and overall cardiovascular health. Their low glycemic index ensures a gradual release of sugar into the bloodstream, making them an excellent dietary option for individuals with diabetes. Given these benefits, exploring the potential of orange-derived compounds as an alternative therapeutic approach for diabetes treatment is a promising research avenue.

This study aims to investigate the inhibitory effects of phytochemicals derived from orange pith on alpha-amylase, an enzyme involved in carbohydrate digestion. By conducting drug-likeness assessments using Lipinski's rule via SwissADME, the study evaluates the suitability of these phytochemicals for therapeutic use. Molecular docking simulations will be employed to explore the binding interactions between specific phytochemicals and alpha-

amylase, providing insights into their potential effectiveness. Through a comprehensive analysis of these interactions, the research seeks to determine whether bioactive compounds from orange pith can serve as viable natural alternatives for diabetes management. This study contributes to the ongoing search for safer and more effective treatments for diabetes, addressing the limitations of conventional anti-diabetic drugs. The researchers' objectives are:

1. Assessment of Drug-likeness

Conduct a drug-likeness evaluation of the primary phytochemical constituents extracted from orange pith by employing Lipinski's rule via SwissADME.

2. Molecular Docking Simulations

Utilize molecular docking simulations to explore and understand the binding interactions between specific phytochemicals from orange pith and alpha-amylase.

3. Analysis of Binding Interactions

Provide insights into the strength and nature of the binding interactions exhibited by the selected phytochemicals when bound to alpha-amylase through comprehensive analysis and visualization.

2. METHODOLOGY

Preparation and Screening Of Orange Pith Phytochemicals

In this study, in silico methods were employed to investigate the potential bioactive compounds in the albedo (white spongy layer) of Citrus sinensis (sweet orange). Using computational models, the chemical constituents of the albedo were virtually identified from databases like PubChem and other phytochemical resources that list compounds found in Citrus species.

The selected compounds were then subjected to molecular docking using the MOLDock algorithm. Docking simulations were performed to predict interactions between the identified albedo compounds and specific target proteins of interest. The proteins were chosen based on their relevance to the biological activity being studied (e.g., antioxidant, anti-inflammatory, or antimicrobial properties).

Preparation of Human Salivary Amylase (RCSB PDB - 1SMD)

The 3D structure of the target protein (RCSB PDB ID: 1SMD) was downloaded as a 3D model from the RCSB Protein Data Bank (https://www.rcsb.org/) in PDB format. After downloading the PDB file, it underwent energy minimization on the Swiss PDB viewer. Then, it was exported to MGL AutodockTools for preparation.

MGL AutodockTools for preparation. Water molecules (HOH1363.A-HOH1572.A) were deleted, and polar hydrogens and Kollman charges were added and evenly distributed throughout the protein (Sharma & Sharma, 2021; Ali et al., 2018). The modified protein file was saved as a PDBQT file to be accessed by Autodock Vina.

Receptor Grid Box Manual Generation

The active binding residues of the RCSB PDB - 1SMD protein were predicted through CASTp (Ali et al., 2018). The receptor grid area was determined using Autodock Vina through MGL AutodockTools. The center of the grid box was positioned according to the active binding site in correspondence to the active residues of the main pocket of the receptor protein; **TRP58, TRP59, TYR62, GLN63, HIS101, LEU162, SER163, LU165, ARG195, ASP197, ALA198, GLU23, HIS299, ASP300, HIS305**. The binding site size was minimized to 80x80x60 Angstrom (Ali et al., 2018) to lessen the probability of the software producing less accurate results and unnecessary binding outcomes. The XYZ values of the coordinates of the center of the grid box (x: 8.398; y: 49.658; z: 20.627), as well as the dimensions of the grid box (80x80x60), were written in a text document for the configuration file with the exhaustiveness level set to 8 and energy range of 3 as per default protocol

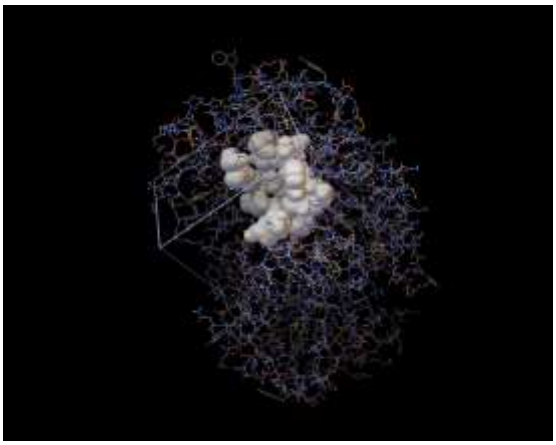

Figure.1 *Receptor grid box visualization through MGL AutodockTools v. 1.5.7*

Molecular Docking Analysis and Simulation

The docking analysis and simulation occurred once the optimized ligands, protein, and configuration files were saved in the same folder. The process occurred through the Command Prompt of Windows 10 and 11 systems. The Autodock Vina (Santos et al., 2019) software for docking was utilized through the computer's Command Prompt. The location was transferred to the folder's directory, where the ligand, protein, and configuration files were stored. Then the code:

"C:\Program Files (x86)\The Scripps Research Institute\Vina\vina.exe" --receptor protein.pdbqt --ligand [ligand.pdbqt] --config [config.txt] --log [log.txt] --out [output.pdbqt]"

was written in the prompt for the computation (Sharma & Sharma, 2021). This docking was done, and the binding affinities of the molecules docked to the protein of diabetes alpha salivary amylase (PDB ID: 1SMD) were given. This process was done ten times for each ligand. The product files of this code were the log files in text format and the output files in PDBQT format for the simulation. After the computation, the output and protein files (in PDBQT format) were exported to PyMol for the 3D simulation. UCSF Chimera, PyMOL, and Ligplot+ were used to visually show the docking process between proteins and ligands.

Scoring and Analysis

The results of the docking computation were saved as a text file in the folder in which the other docking files were located. The log text file displayed the binding affinity levels of the different phytocompounds to the proteins. A score function evaluates the conformation and orientation of the ligand in the protein's binding site and measures binding affinity, which is the strength of the interaction between a ligand and a protein. A more negative binding affinity score indicates a stronger interaction between the ligand and the protein and, thus, a higher likelihood of successful binding (Pantsar & Poso, 2018; Torres et al., 2019). Only the results with the ligand situated inside the main pocket of the protein were considered competent data. Out of the ten docking attempts, the output that showed the lowest binding affinity value was chosen as the representative data for the docking interaction for each ligand, given that the compound is situated inside the protein's main pocket. The lesser the value of binding affinity, the stronger the interaction between the protein and the ligand (Ali et al., 2018), as it indicates a higher energy release upon binding, resulting in a more stable complex between molecules (Seo et al., 2021), therefore a higher inhibition potential for protein-protein interactions.

The structure-based virtual screening method (Ali et al., 2018) was used for the visual analysis, and PyMol was used to see the 3D outputs of the protein-ligand docking. The hydrogen bonds and hydrophobic interactions were viewed using Ligplot+. Scoring functions are important components in molecular docking for structure-based drug discovery. Traditional scoring functions, generally empirical- or force field-based, are robust and have proven to be useful for identifying hits and lead optimizations (Yanjie et al, 2022). The interacting residues are also evaluated after docking through Ligplot+.

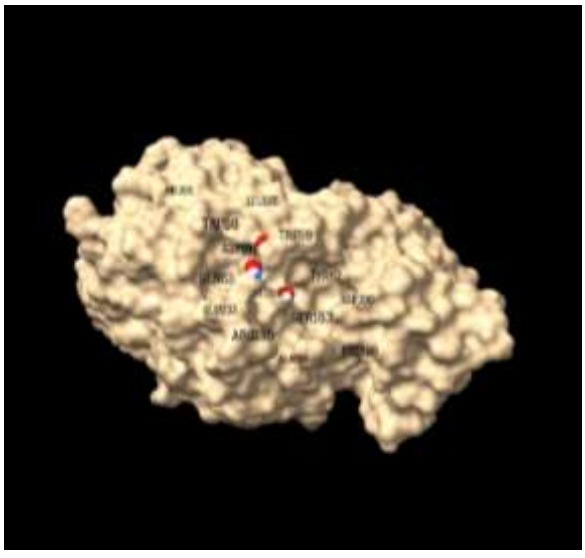

Fig 2.

The residues of the binding sites of the protein that interact with the ligand will be compared to the residues of the protein that naturally interact with the residues of 1MSD: TRP58, TRP59, TYR62, GLN63, HIS101, LEU162, SER163, LEU165, ARG195, ASP197, ALA198, GLU233, HIS299, ASP300, HIS305. The binding affinity levels of ligands interacting with the 1MSD protein can effectively rank these ligands based on their potential to inhibit immune responses. By binding to the protein, ligands may disrupt signaling pathways, particularly those involved in immune activation. This mechanism is crucial for developing therapeutic agents aimed at modulating immune responses, as understanding these interactions enhances drug design and efficacy. Metformin functioned as the positive control for this study. Due to its outstanding efficacy and safety profile, physicians have widely used Metformin as a aid to lower the blood sugar of Diabetes.

Methodological Framework

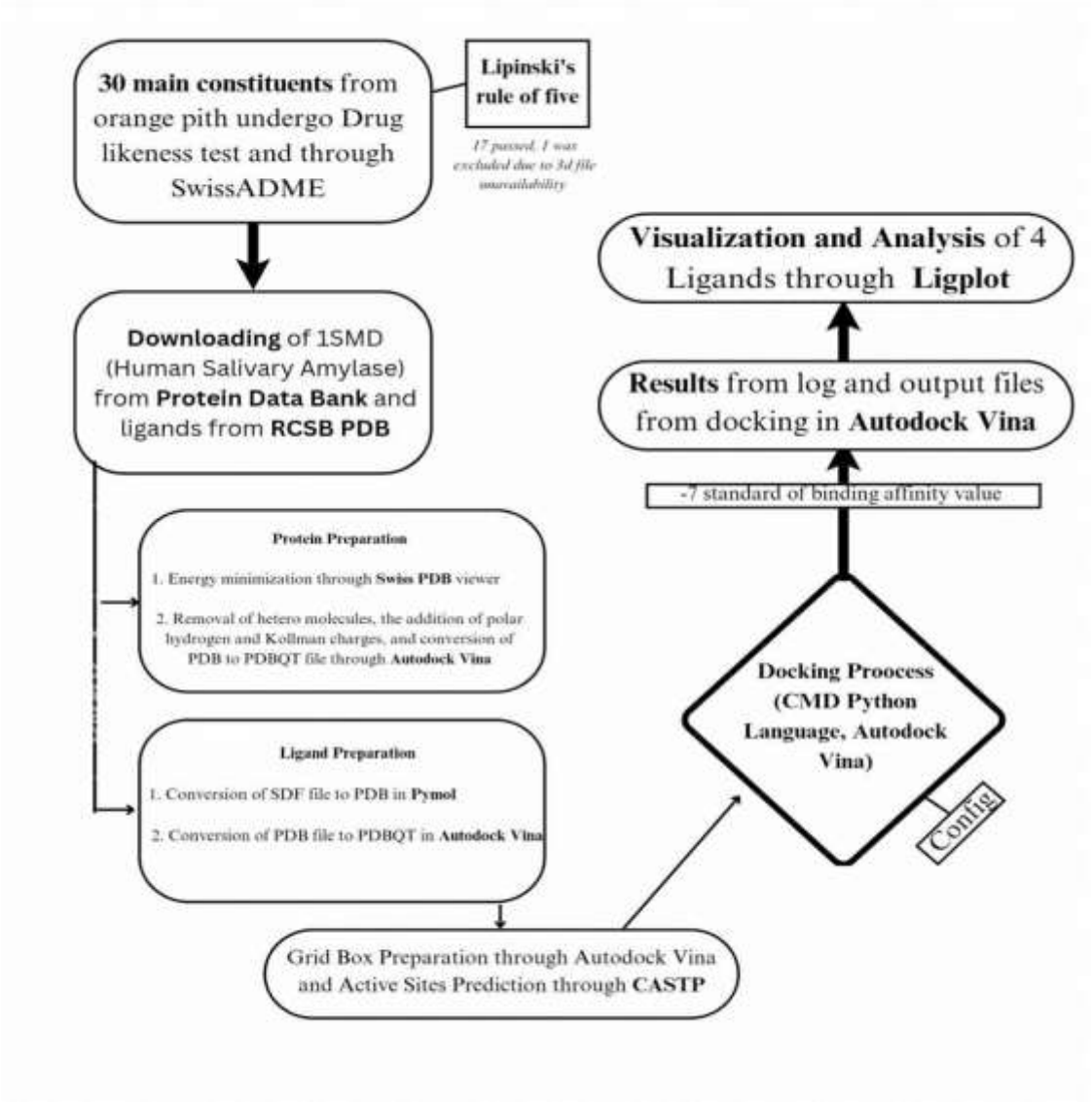

Fig 3. *Methodological Framework of the study*

3. RESULTS AND DISCUSSIONS

This study aimed to determine and analyze the 30 primary phytochemical components obtained from *Citrus sinensis albedo* targeting attachment to a-amylase active binding sites. The 30 phytochemicals were subjected to SwissADME following the Lipinski rule, where the physicochemical properties of the phytochemical ligands were evaluated before the molecular docking procedure. Among the 31 phytochemicals, 17 were found to have passed Lipinski's rule of 5.

Table 1: *Phytochemicals found in C. sinensis albedo*

a	***b***	***c***	***d***	***e***	***f***
Stigmasterol	412.69	1	1	5.85	1
Nobiletin	402.39	0	8	5.35	1
Tangeretin	402.39	0	8	5.70	1

Rutin	610.53	5	9	5.52	3
Luteolin	286.24	3	6	1.43	0
Apigenin	270.23	2	6	1.53	0
Catechin	290.27	5	6	0.58	1
Epicatechin	293.47	3	5	0.63	0
Myricetin	318.25	6	8	1.68	1
Gallotannin	562.26	6	9	5.62	3
Scopoletin	192.70	3	4	3.27	0
D-limonene	136.23	0	1	4.23	1
P-coumaric acid	164.19	3	4	1.24	0
Ferulic acid	193.18	4	5	1.59	0
Caffeic acid	180.14	3	4	0.70	0
Chlorogenic acid	354.31	6	7	-0.85	1
Beta-sitosterol	414.72	1	3	6.5	2
Beta-carotene	318.24	6	1	-1.57	1
Naringenin	272.25	3	5	1.71	0
Linoleic acid	280.46	2	2	3.5	0
Hexadecane	272.50	0	0	6.25	1
Gallic acid	170.12	3	5	0.36	0
Protocatechuic acid	154.32	3	4	0.90	0
4-hydroxybenzoic acid	138.12	5	4	1.50	1
Citric acid	210.14	4	7	-1.30	0

Ascorbic acid	176.32	4	6	-1.50	0
L-malic acid	134.09	4	5	-0.25	0
Quercetin	302.24	5	7	1.60	1
Vanillic acid	182.17	4	4	1.29	0
Syringic acid	212.20	3	5	1.45	0
Salicylic acid	138.22	2	3	1.20	0

***a** = Ligands; **b** = Molecular Weight (g/mol, <**500 Da**); **c** = Number of Hydrogen bond donors (<**5**); **d** = Number of Hydrogen bond acceptors (<**10**); **e** = M Log $P_{o/w}$ (**≤4.15**); **f** = Number of Violations (<**1**)*

The table presented the physicochemical properties of the 31 ligands as determined by SwissADME and Lipinski's Ro5. Stigmasterol, Nobiletin, Tangeretin, Rutin, Catechin, Quercetin, Myricetin, Gallotannin, D-limonene, Chlorogenic acid, Beta-sitosterol, Beta-carotene, Hexadecane, 4-hydroxybenzoic acid had violations. However, the infraction was deemed acceptable, and the subsequent ligands' drug-like characteristics were nonetheless acknowledged

Notably, one of the 31 phytochemicals was unavailable due to the absence of a three-dimensional (3D) structure, which resulted in the reduction of the phytochemicals under consideration to 30. The table presented the physicochemical properties of these 30 ligands as determined by SwissADME and Lipinski's Ro5. Stigmasterol, Nobiletin, Tangeretin, Rutin, Catechin, Quercetin, Myricetin, Gallotannin, D-limonene, Chlorogenic acid, Beta-sitosterol, Beta-carotene, Hexadecane, and 4-hydroxybenzoic acid had violations. The remaining phytochemicals were subsequently docked with the control variables on PDB ID: 1MSD. A total of 10 repetitions were executed to ensure both consistency and precision. Afterward, the docking procedure generated ten unique outputs and their corresponding reports; the output with the lowest binding affinity value was recorded for each iteration.

Scoring Functions

Pymol was used to visualize and identify the positions of the ten docking attempts that occupied the binding pocket. A standardized -7.0 affinity value was given to pinpoint the phytochemicals having a higher chance of interacting with the 1MSD protein. This process narrowed down the number to 17. Each ligand conformation (1st pose) was integrated into PDB files, generating 17 ligand complexes subjected to visual analysis.

***Table 2:** 16 Phytochemicals with -7.0 and Lower Binding Affinity Values*

a	***b***	***c***	***d***
Luteolin	5280445	-9.0	Output no.3
Citric acid	311	-5.7	Output no.1
Apigenin	5280443	-8.8	Output no.5
Scopoletin	5280460	-6.3	Output no. 2
Naringenin	439246	-9.8	Output no. 4
Gallic acid	370	-6.3	Output no.3
Epicatechin	72276	-8.7	Output no.1
L-malic acid	311	-4.7	Output no. 7

Ascorbic acid	54670067	-5.9	Output no.2
Vanillic acid	8468	-5.5	Output no.1
Syringic acid	10742	5.6	Output no.3
Salicylic acid	10742	-5.8	Output no.5
P-coumaric	637542		Output no.7
Ferulic acid	445858	-6.7	Output no.3
Caffeic acid	689043	-6.8	Output no.1
Linoleic acid	5280450	-5.1	Output no.4

***a** = phytochemical name; **b** = CID from PUBCHEM (<**5**); **c** = binding affinity values; **d** = output number*

Among the phytochemicals, the ligands with the highest binding affinity score was Naringenin, followed by Luteolin and Apigenin, Epicatechin, are the leading ligands according to this ranking. The ligands exhibited a diverse array of remarkable binding affinities, wherein Naringemin exhibited the highest value of -9.8 kcal/mol, closely followed by Luteolin at -9.0 kcal/mol. The binding affinities of the remaining ligands, including Apigeninl and Epicatechin, varied between -8.8 and -8.7 kcal/mol.

Protein and Ligand Interactions between interacting residues

Diabetes, a chronic metabolic disorder characterized by high blood sugar levels, results from defects in insulin signaling and glucose metabolism. Insulin, a key hormone produced by the pancreas, binds to insulin receptors (IR) on host cells, primarily in muscle, liver, and adipose tissues. This interaction is crucial for facilitating glucose uptake and maintaining blood sugar homeostasis. The binding of insulin to its receptor occurs through specific domains that trigger downstream signaling pathways, enabling glucose transport into cells. This research looks at 4 phytochemicals: Naringenin, Apigenin, Luteolin, Epicatechin. Anong these, Naringenin showed the best results in molecular docking studies with the target protein: Human Salivary 1MSD which are involved in

Table 3: *Ligplot Analysis of the 4 Phytochemicals*

a	**b**	**c**
Naringenin	Asp197	Lys352(A)
	[2.93]	Tyro2(A)
	Arg195	Leu165(A)
	[3.34]	Trp58(A)
	Glo233	Trp59(A)
	[3.22]	His299(A)
	Asp300	
	[3.06]	
	His305	
	[3.23]	
	Asp356	
	[2.94]	
	Gln63	
	[3.25]	

Apigenin	Gln63 [3.18] Asp197 [2.77] Glu233 [3.14]	Trp59(A) Tyro62(A)Leu165(A Asp300(A His299(A) Trp58(A)
Luteolin	G1n63 [3.12] Arg195 [3.25] Asp197 [2.99] Glu233 [3.11]	Leu165(A Asp300(A His299(A) Trp58(A) Tyr62(A) Trp59(A)
Epicatechin	Trp59 [2.70] Asp197 [2.97]	Gln63(A) Leu165(A Trp58(A) Asp300(A Tyro62(A)

a = phytochemical name; **b** = hydrogen bonds with interacting residues; **c** = Hydrophobic interactions *with interacting residues;* ***bold*** = *binding sites of insulin (IR)*

To understand Naringenin, we looked at its interaction with important residues in these proteins. For protein Human Salivary 1MSD some key residues include Asp 197, Arg 195, Glo 233, Asp 300, His 305, Asp 356, and Gln 63. All three phytochemicals had a binding affinity of less than -7 kcal/mol during the docking studies.

According to this ranking, the top three ligands were naringenin, apigenin, and luteolin. The ligands demonstrated a wide range of impressive binding affinities, with Naringenin consistently exhibiting the highest value of -9.8;kcal/mol, followed by Luteolin which had a value of -8.8 kcal/mol, and Apigenin is the third leading factor, with a value of -9.0.

Binding affinity and hydrogen bonds are crucial in molecular docking as they significantly influence the stability and specificity of protein-ligand interactions. Hydrogen bonds enhance binding affinity by facilitating optimal donor-acceptor pairings, which can reduce competition from water molecules, thus stabilizing the ligand-receptor complex (Zhang et al., 2016). Moreover, accurate evaluation of hydrogen bond potential is essential for predicting binding affinities during drug design, allowing for effective virtual screening of compounds (Huang et al., 2019). Understanding these interactions aids in developing better therapeutic agents through improved molecular docking techniques (Kumar et al., 2018).

Top Three Phytochemicals with highest binding affinity and prominent interacting residues

Naringenin was the most effective among the three phytochemicals. Its interactions with the residues of the three proteins showed strong binding. In the protein Human Salivary 1MSD, it had hydrophobic interactions in Lys352(A), Tyro2(A), Leu165(A), Trp58(A), Trp59(A), His299(A). It specifically interacted with binding sites Asp197 (A), Arg195 (A), Glo233 (A), Asp300 (A), His305 (A), Asp356 (A), Gln63 (A). Luteolin was the second one that was effective. It had hydrophobic interactions in Leu165(A), Asp300(A), His299(A), Trp58(A), Tyr62(A), Trp59(A). And lastly Apigenin was the third one that was effective, it had hydrophobic interactions in Trp59(A), Tyro62(A), Leu165(A), Asp300(A), His299(A), Trp58(A).

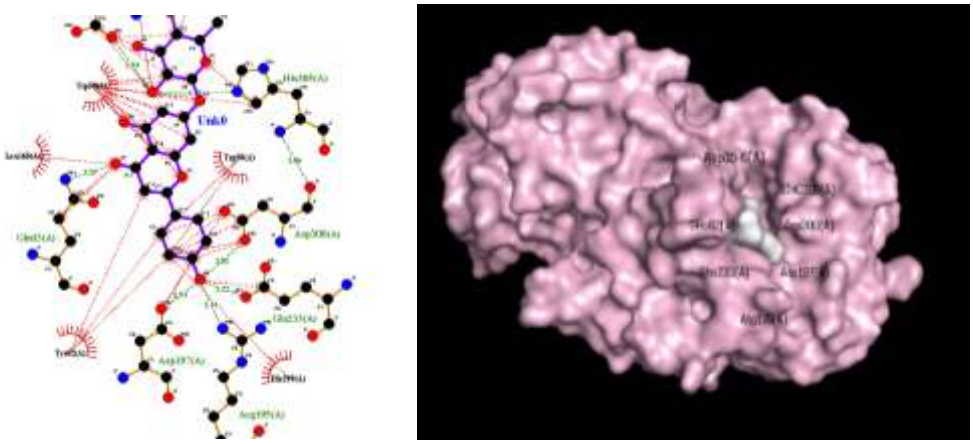

Figure 4. *Naringenin complex. Chimera 3D visualization (right); Ligplot+ hydrogen bonds and hydrophobic interactions 2D visualization (left).*

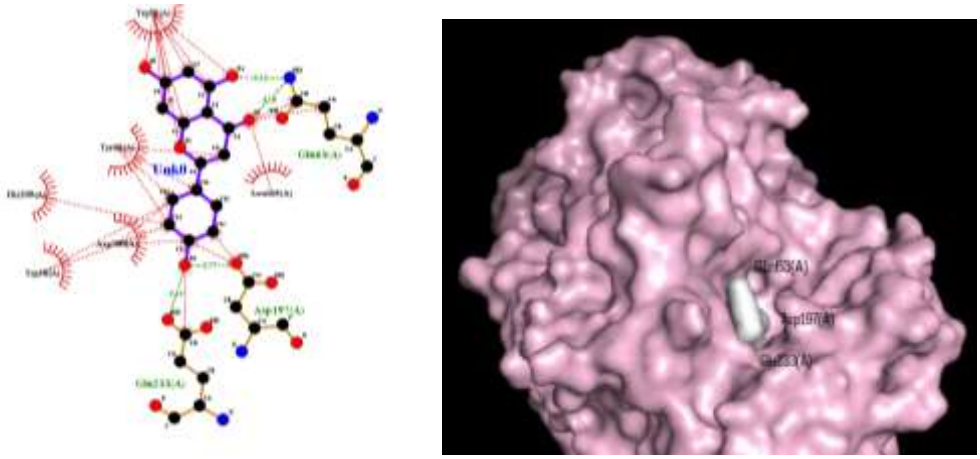

Figure 5*. Apigenin complex. Chimera 3D visualization (right); Ligplot+ hydrogen bonds and hydrophobic interactions 2D visualization (left).*

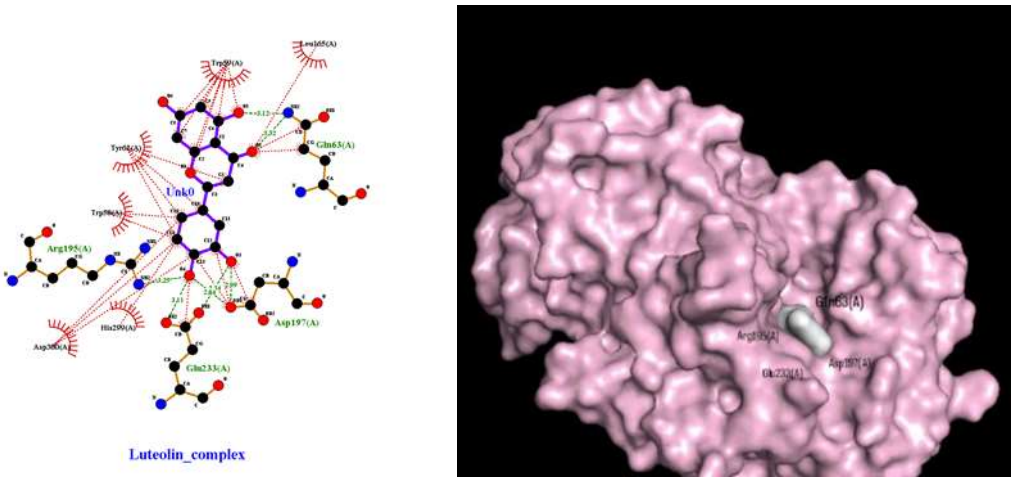

Figure 6. *Luteolin complex. Chimera 3D visualization (right); Ligplot+ hydrogen bonds and hydrophobic interactions 2D visualization (left).****Other Phytochemicals with notable interacting residues***

Epicatechin demonstrated noteworthy interactions with key residues in the target protein, with a binding affinity score of (-8.7kcal/mol) on the protein, Epicatechin formed stable interactions with Human Salivary 1MSD hydrophobic interactions in Gln63(A), Leu165(A), Trp58(A), Asp300(A), Tyro62(A). There were also hydrogen bonds in Trp59 (A) at 2.70, Asp197 (A) at 2.97. Overall, the interactions of these phytochemical showed that it also has a potential to be able to lower the blood sugar of Diabetes.

Comparing Albedo Extract with Metformin as a Positive Control.

In this study, Metformin was used as the positive control to evaluate the potential control of Albedo Extract as a help to lower the blood sugar of Diabetes. Metformin is a widely prescribed medication for managing type 2 diabetes and gestational diabetes. It works by lowering blood sugar levels through improved insulin sensitivity and reduced glucose production in the liver. Common side effects include gastrointestinal issues, and it may also contribute

to modest weight loss, making it suitable for overweight patients with diabetes. Metformin is often the first-line treatment due to its effectiveness and safety profile NHS.(n.d) (2020). In the molecular docking results, it showed that Albedo Extract exhibited a binding affinity lower than -7 kcal/mol, indicating a strong interaction with the target receptor. In comparison, Metformin, despite being known as a common drug used to lower the blood sugar of diabetes, showed a weaker binding affinity, suggesting reduced stability in receptor binding. These findings suggest that Albedo Extract may offer stronger and more stable receptor binding, positioning it as a help to lower the blood sugar of diabetes.

Table 4: 4 *Phytochemicals found in metformin*

a	***b***	***c***	***d***	***e***	***f***
Quercetin	302.24	5	7	2.07	0
Galegine	286.27	3	6	2.58	1
Ferulic acid	193.18	4	5	1.59	0
Caffeic acid	180.14	3	4	0.70	0

***a** = Ligands; **b** = Molecular Weight (g/mol, <**500 Da**); **c** = Number of Hydrogen bond donors (<**5**); **d** = Number of Hydrogen bond acceptors (<**10**); **e** = M Log $P_{o/w}$ (**≤4.15**); **f** = Number of Violations (<**1**)*

Conclusions and Recommendations

Noteworthy results from the in silico docking analysis of various phytochemicals targeting the alpha-amylase enzyme (PDB ID: 1MSD) have been obtained. Among the compounds docked and analyzed, naringenin ranked highest and exhibited the strongest binding affinity for alpha-amylase, showcasing its potential as an effective inhibitor. All examined phytochemicals demonstrated excellent binding affinities and interacted with at least one or more crucial residues of the alpha-amylase enzyme.

The collective results of this study strongly suggest that each of the analyzed phytochemicals has the potential to act as alpha-amylase inhibitors in an in silico environment, positioning them as promising candidates for further exploration through in vitro assays. It is crucial to conduct in vitro assays using relevant cell lines to validate these findings, providing valuable insights into their inhibitory capabilities and enabling an evaluation of efficacy in a biological context. Subsequent investigations should prioritize dose-response studies to identify the most effective inhibitory concentrations, ensuring the development of safe and effective diabetes management strategies.

Employing molecular techniques, such as biochemical assays or molecular dynamics simulations, will facilitate a deeper understanding of the specific interactions, binding kinetics, and structural changes induced by the identified phytochemicals on the alpha-amylase enzyme.

Conducting comprehensive safety profiling, encompassing off-target interactions, cytotoxicity, and potential adverse effects, is crucial before initiating drug development. Additionally, conducting animal model studies will yield significant insights into the compounds' pharmacokinetics, biodistribution, and overall efficacy within a complex biological system.

Examining the correlation between the structure and activity of the identified phytochemicals is essential for developing derivatives with stronger binding and improved pharmacological properties. Collaborating with experts to translate in silico findings into practical treatments for diabetes enhances our understanding and contributes to the global knowledge of diabetes management. This collaboration fosters data exchange and insights, promoting further progress in the field.

In summary, the in silico docking analysis provides an encouraging initial stage and a lead to further computer-aided drug design aimed at managing diabetes. The suggestions above emphasize the importance of conducting thorough experimental validation, safety evaluations, and additional mechanistic investigations to advance the development of effective therapies targeting alpha-amylase, particularly in an in vitro setting.

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References:

Sapra, A., & Bhandari, P. (2023, June 21).

Diabetes. StatPearls - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK551501/

Zhang, J., Zhang, L., Lai, C., Liang, Y., Gao, L., Kaliaperumal, K., & Jiang, Y. (2022).

Nutraceutical potential of navel orange peel in diabetes management: The chemical profile, antioxidant, α-glucosidase inhibitory and antiglycation effects of its flavonoids. Food Bioscience, 49, 101943. https://doi.org/10.1016/j.fbio.2022.101943

Roglic, G. (2016).

WHO global report on diabetes: A summary. International Journal of Noncommunicable Diseases, 1(1), 3–8. https://doi.org/10.4103/2468-8827.184853

Saikia, S., & Bordoloi, M. (2019).

Molecular docking: Challenges, advances, and its use in drug discovery perspective. *Current Drug Targets, 20*(5), 444–456. https://www.ingentaconnect.com/content/ben/cdt/2019/00000020/00000005/art00005

Karle, P. P., Dhawale, S. C., Navghare, V. V., & et al. (2021).

Optimization of extraction conditions and evaluation of Manilkara zapota (L.) P. Royen fruit peel extract for in vitro α-glucosidase enzyme inhibition and free radical scavenging potential. Future Journal of Pharmaceutical Sciences, 7, 151. https://doi.org/10.1186/s43094-021-00305-4

Morales-Brown, L. (2023, July 25).

Oranges for diabetes. Medical News Today. https://www.medicalnewstoday.com/articles/oranges-for-diabetesMarnie. (2019).

Are there health benefits in the orange pith?https://www.whatishealth.net/my-story-and-bio

Morris, G. M., & Lim-Wilby, M. (2008).

Molecular docking. Methods in Molecular Biology, 365–382. https://doi.org/10.1007/978-1-59745-177-2_19

Priya, E. S., Selvan, P. S., & Arshad, A. S. (2018).

Analysis of *in vitro* and *in silico* anti-hyperglycaemic action of bioflavonoids isolated from different citrus peels. *Current Science*, *115*(10), 1928–1937. https://www.jstor.org/stable/26978524

Favela-Hernández, J. M. J., González-Santiago, O., Ramírez-Cabrera, M. A., Esquivel-Ferriño, P. C., & Camacho-Corona, M. D. R. (2016).

Chemistry and pharmacology of Citrus sinensis. Molecules, 21(2), 247. https://doi.org/10.3390/molecules21020247

Kanaze, F. I., Termentzi, A., Gabrieli, C., Niopas, I., Georgarakis, M., & Kokkalou, E. (2009).

The phytochemical analysis and antioxidant activity assessment of orange peel (Citrus sinensis) cultivated in Greece-Crete indicates a new commercial source of hesperidin. Biomedical Chromatography, 23(3), 239–249. https://doi.org/10.1002/bmc.1090

Escobedo-Avellaneda, Z., Gutiérrez-Uribe, J., Valdez-Fragoso, A., Torres, J. A., & Welti-Chanes, J. (2014).

Phytochemicals and antioxidant activity of juice, flavedo, albedo and comminuted orange. Journal of Functional Foods, 6, 470-481. https://doi.org/10.1016/j.jff.2013.11.013

Qurtam, A. A., Mechchate, H., Es-safi, I., Al-Zharani, M., Nasr, F. A., Noman, O. M., Aleissa, M., Imtara, H., Aleissa, A. M., Bouhrim, M., & others. (2021).

Citrus flavanone narirutin, in vitro and in silico mechanistic antidiabetic potential. Pharmaceutics, 13(11), 1818. https://doi.org/10.3390/pharmaceutics13111818

Hassan, M. A., Elmageed, G. M. A., El-Qazaz, I. G., El-Sayed, D. S., El-Samad, L. M., & Abdou, H. M. (2023).

The synergistic influence of polyflavonoids from Citrus aurantifolia on diabetes treatment and their modulation of the PI3K/AKT/FOXO1 signaling pathways: Molecular docking analyses and in vivo investigations. Pharmaceutics, 15(9), 2306. https://doi.org/10.3390/pharmaceutics15092306

Zheng, L., Meng, J., Jiang, K., Lan, H., Wang, Z., Lin, M., Li, W., Guo, H., Wei, Y., & Mu, Y. (2022).

Improving protein-ligand docking and screening accuracies by incorporating a scoring function correction term. Briefings in Bioinformatics, 23(3), bbac051. https://doi.org/10.1093/bib/bbac051

López-Téllez, G., Vílchis Néstor, A. R., & others. (2018).

Green and sustainable synthesis of metal nanoparticles using orange peel pith. In *Green Metal Nanoparticles* (pp. 214–229). Wiley Online Library. https://doi.org/10.1002/978111

Elavarasan, M., & Premalatha, A. (2019).

A review: Nutrient deficiencies and physiological disorders of citrus. Journal of Pharmacognosy and Phytochemistry, 8(4), 1705-1708. https://www.phytojournal.com/archives?year=2019&vol=8&issue=4&ArticleId=9192

Santos, L. H. S., Ferreira, R. S., & Caffarena, E. R. (2019).

Integrating molecular docking and molecular dynamics simulations. *Methods in Molecular Biology (Clifton, N.J.)*, *2053*, 13–34. https://doi.org/10.1007/978-1-4939-9752-7_2

Multari, S., Licciardello, C., Caruso, M., & others. (2021).

Flavedo and albedo of five citrus fruits from Southern Italy: Physicochemical characteristics and enzyme-assisted extraction of phenolic compounds. *Food Measurement*, *15*, 1754–1762. https://doi.org/10.1007/s11694-020-00787-5

Muzykiewicz, A., Zielonka-Brzezicka, J., & others. (2019).

The antioxidant potential of flesh, albedo, and flavedo extracts from different varieties of grapefruits. *Acta Scientiarum Polonorum Technologia Alimentaria*, *18*(4), 401–409. https://www.food.actapol.net/volume18/issue4/abstract-9.html